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Light chain proximal tubulopathy

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CASE PRESENTATION

A 55-year-old Caucasian woman presented to her primary care physician with complaints of progressive fatigue for several months, dyspnea with minimal exertion, loss of appetite, 15-pound weight loss, and recurrent low-grade fevers. Past medical history was significant only for migraine headaches. The patient was taking no prescription or over-the-counter medications. There was no history of environmental toxin exposure, recent travel, smoking, excess alcohol consumption, or use of illicit drugs. She was empirically treated with a course of esomeprazole and amoxicillin.

The patient returned 2 weeks later and reported no improvement in her symptoms. Laboratory studies revealed anemia, thrombocytopenia, hypercalcemia, and acute renal failure. The patient was admitted for further evaluation.

Upon admission, physical examination revealed a well nourished but pale female in no acute distress. Her blood pressure was 156/70 mm Hg, pulse 96 bpm, temperature 98.6 F, respiratory rate 20 breaths/min, and pulse oximetry 100% on room air. Cardiac and pulmonary examinations were unremarkable. Abdominal examination revealed splenomegaly with a palpable liver edge 1–2 cm below the right costal margin. Laboratory testing (Table 1) was notable for a hemoglobin of 6.1 g/dl (normal range, 11.0–15.0 g/dl), platelet count 112 K/mm^3 (nl 150–400 K/mm^3), creatinine 3.7 mg/dl, BUN 34 mg/dl, and calcium 12.4 mg/dl (nl 8.5–10.1 mg/dl). Liver function tests, coagulation studies, and parathyroid hormone levels were normal. Urinalysis revealed 1+ protein, 1+ glucose, and trace blood. Negative serologies included anti-glomerular basement membrane antibody, proteinase-3 anti-neutrophil cytoplasmic antibodies, and myeloperoxidase anti-neutrophil cytoplasmic antibodies. Serum complement levels were mildly elevated with C3 179.0 mg/dl (nl 75–135 mg/dl) and C4 41.9 mg/dl (nl 9–36 mg/dl). Serum protein electrophoresis with immunofixation showed no monoclonal protein. A bone marrow biopsy revealed normocellular marrow with no evidence of lymphoma or a plasma cell dyscrasia. A non-contrast computed tomography scan of the chest, abdomen, and pelvis was notable for splenomegaly (18 × 15 × 10 cm) with a hypodense area measuring 7 × 7 × 4 cm and mild hepatomegaly. The kidneys measured 11.7 and 12 cm in length by ultrasound, without evidence of obstruction. A skeletal survey showed no abnormalities.

The patient was treated with hydration and a single dose of pamidronate. At the time of discharge 6 days later, her creatinine had fallen to 1.9 mg/dl, and her calcium had normalized.

The patient was seen in nephrologic consultation 1 week after discharge, at which time her creatinine had declined to 1.5 mg/dl. Computed tomography scan was repeated with contrast and revealed a 19 × 16 × 10 cm spleen with an ill-defined 11 × 11 × 13 cm mass with areas of probable necrosis. In light of the absence of a clear indication for splenectomy, a renal biopsy was performed to determine the cause of the patient's persistent renal dysfunction.

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RENAL BIOPSY FINDINGS

The renal biopsy was processed for light microscopy, immunofluorescence (IF), and electron microscopy, according to standard techniques. Sampling for light microscopy included two cores of renal cortex containing 14 glomeruli, one of which was globally sclerotic. Glomeruli appeared histologically unremarkable. Proximal tubules displayed mild degenerative changes characterized by luminal ectasia, cytoplasmic simplification with vacuolization, and prominent nucleoli. The degenerative changes in proximal tubules were accompanied by intracellular inclusions, which appeared pale with the hematoxylin and eosin and PAS stains and multifocally formed cleft-like spaces (Figure 1a). There was mild interstitial inflammation composed of mainly lymphocytes and rare foci of mild tubulitis. There was moderate tubular atrophy and

Table 1 | Laboratory data at initial hospital admission

Laboratory tests	Results (normal values)
Hemogram	
WBC	5.58 (4.0–10.0 K/mm^3)
Hemoglobin	6.1 (11.0–15.0 g/dl)
Platelet count	112 (150–400 K/mm^3)
Sodium	138 (136–145 meq/l)
Potassium	3.3 (3.5–5.1 meq/l)
Chloride	102 (102–110 meq/l)
CO_2	27 (22–29 meq/l)
Glucose	104 (70–110 mg/dl)
Calcium	12.4 (8.5–10.1 mg/dl)
BUN	34 (7–23 mg/dl)
Creatinine	3.7 (0.6–1.3 mg/dl)
LDH	247 (100–190 U/l)
Total protein	6.3 (6.4–8.2 g/dl)
Albumin	2.9 (3.4–5.0 g/dl)
Haptoglobin	268 (30–178 mg/dl)
Iron	26 (35–150 mcg/dl)
Iron binding capacity	235 (250–450 mcg/dl)
Iron saturation%	11 (15–55%)
PTH	4.79 (11–67 pg/ml)
PTH-RP	$<$0.3 (0.0–1.5 pmol/l)
Vitamin B12	520 (193–982 pg/ml)
Folate	13.5 (3.0–17.0 ng/ml)
Vit D 1,25	91.3 (15.9–55.6 pg/ml)
Vit D 25-OH	56.3 (32.0–100.0 pg.ml)

BUN, blood urea nitrogen; LDH, lactate dehydrogenase; PTH, parathyroid hormone: WBC, white blood cells.

interstitial fibrosis involving approximately 40% of the cortex sampled. Vessels exhibited mild arteriosclerosis. Congo red staining for amyloid was negative.

Sampling for IF consisted of 5 glomeruli and exhibited no significant glomerular positivity for IgG, IgM, IgA, C3, C1, fibrinogen, albumin, or κ or λ light chains. There were rare tubular casts, which stained similarly for IgA, κ, and λ. Following review of the ultrastructural findings, immunoflourescence staining was repeated following pronase digestion and revealed intracellular needle-shaped crystals in proximal tubules which stain positive for κ and negative for λ (Figure 1b).

The predominant abnormalities seen on ultrastructural evaluation involved the proximal tubules. In addition to tubular degenerative changes, proximal tubules contained abundant intracellular and focal intraluminal crystalline inclusions. The inclusions had a fibrillar appearance and formed parallel arrays and groupings. A minority of the inclusions were found to lie in membrane-bound structures (Figure 1c and d). Glomeruli exhibited no significant ultrastructural abnormalities. There was no significant foot process effacement and no electron dense deposits were apparent.

Renal biopsy diagnosis: light chain proximal tubulopathy, κ-type (a.k.a. Light chain Fanconi syndrome)

Clinical follow-up No. 1. Following receipt of the renal biopsy results, serum free light chain testing was performed and showed a free κ level of 25.20 (nl 3.30–19.40 mg/l), free λ of 28.10 (nl 5.71–26.30 mg/l), and a normal κ/λ ratio of 0.9 (nl 0.26–1.65). Urine protein electrophoresis with immunofixation showed a monoclonal κ Bence Jones protein. Light chain Fanconi syndrome (LCFS) is mainly associated with multiple myeloma (MM), which does not typically involve the spleen. Nonetheless, in light of the presence of a monoclonal urine spike and the renal biopsy findings, splenectomy was performed.

SPLENECTOMY FINDINGS

Due to direct extension of the splenic mass into the distal pancreas, combined splenectomy with distal pancreatectomy was performed. Grossly, the splenic parenchyma was almost entirely replaced by a yellow-grey soft mass with a necrotic center. The mass extended into the tail of the pancreas and into peripancreatic adipose tissue.

Microscopic evaluation of the splenic and distal pancreatic mass showed a diffuse, dense sheet-like infiltrate of pleomorphic medium- to large-sized lymphocytes and zones of coagulative necrosis. The lymphocytes exhibited round to ovoid vesicular nuclei with variably multiple small or single prominent nucleoli. In a few areas, the lymphocytes showed plasmacytoid morphology (Figure 2a). Scattered tingible-body macrophages were present. The neoplastic cells expressed CD20 (Figure 2b), Pax-5, and CD79a (B-cell markers), BCL2, and subsets showed either BCL6 or MUM1 expression (markers of germinal center B cells and activated lymphocytes or plasma cells, respectively). Nuclear staining for p53 was observed in 30% of cells. The cells showed a high proliferation index (80%) on staining for Ki-67/MIB1. The neoplastic cells did not express CD3 or CD5 (T-cell markers), CD10, CD23, CD138 (a mature plasma cell marker), or CD43. *In situ* hybridization for immunoglobulin light chain mRNA showed cytoplasmic κ light chain restriction (Figure 2c and d). No EBER positive cells were seen by *in situ* hybridization. The morphology and phenotype were consistent with a diffuse large B-cell lymphoma, 'activated' or non-germinal center type. The variability in cell size and p53 expression raised the possibility of transformation from a prior low-grade lymphoma such as a primary splenic marginal zone lymphoma or a lymphoplasmacytic lymphoma, but no distinct low-grade component was identified.

SPLENECTOMY DIAGNOSIS

Diffuse large B-cell lymphoma with plasmacytoid features

Clinical follow-up No. 2. Following splenectomy, the patient's creatinine declined over 4 days from 1.6 to 1.2 mg/dl. Over the following 3 weeks, the creatinine declined further to 0.8 mg/dl, at which point the patient began chemotherapy. Four months later, following 6 cycles of chemotherapy with CHOP (cyclophosphamide, adriamycin, vincristine, and prednisolone) and Rituximab, the patient has a creatinine of 1.0 mg/dl (Figure 3). A follow-up positron emission tomography scan showed resolution of a previous area of positivity in the left iliac crest that was discovered in the initial staging of the diffuse large B-cell lymphoma.

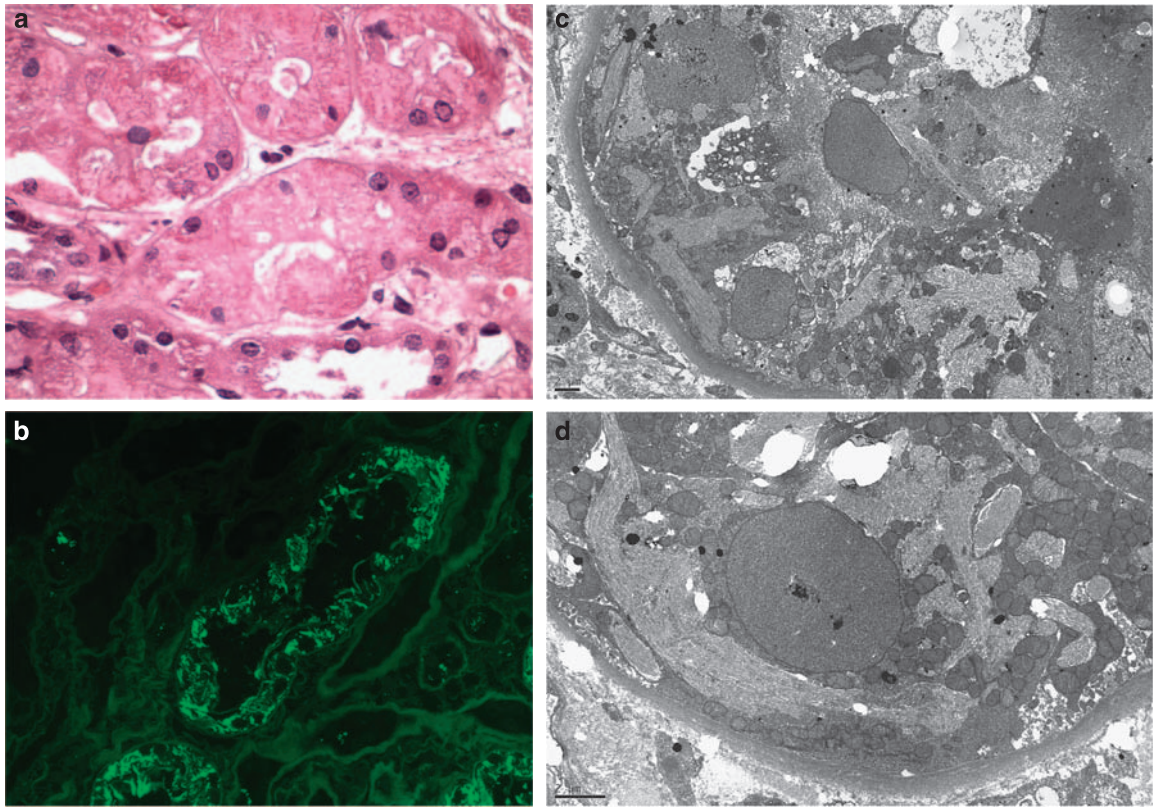

Figure 1 | Renal biopsy findings. (**a**) High power view of proximal tubules reveals pale-staining intracytoplasmic crystals (hematoxylin and eosin, ×600). (**b**) Immunofluorescence following pronase-digestion reveals intracellular crystals within proximal tubules which stain positively for κ light chain. Staining for λ light chain is negative (×400). (**c**) On ultrastructural evaluation, proximal tubular cells contain intracytoplasmic crystalline inclusions (×4000). (**d**) On high power inspection, the intracytoplasmic inclusions have a fibrillar appearance and form parallel arrays. A minority of the inclusions lie in membrane-bound structures (×8000).

DISCUSSION

Light chain proximal tubulopathy, more commonly referred to as light chain Fanconi syndrome (LCFS), is a rare pattern of renal disease that occurs in the setting of dysproteinemia. LCFS shares similarities with the more common entity of myeloma cast nephropathy in that both conditions are characterized by tubular injury with crystalline deposits of monoclonal light chains. In LCFS, the crystalline deposits are intracellular and confined to proximal tubules, as opposed to the intraluminal, distal tubular casts seen in myeloma cast nephropathy.[1] As a result of crystal accumulation in proximal tubules, patients with LCFS exhibit features of Fanconi syndrome (i.e., proximal tubular dysfunction), including normoglycemic glycosuria, aminoaciduria, uricosuria, hyperphosphaturia (with hypophosphatemia), and type II renal tubular acidosis. In the case reported herein, testing for Fanconi syndrome was not specifically performed and as such, the terminology of 'light chain proximal tubulopathy' is utilized. Nonetheless, the patient was noted to have glycosuria in the setting of normal serum glucose.

LCFS is often a difficult diagnosis to establish based on limited awareness of the entity, its relatively indolent presentation in the majority of cases, and the subtle light microscopic findings. Light microscopy typically reveals non-specific findings of acute and chronic tubulointerstitial nephropathy. The cytoplasm of proximal tubules contains pale needle-shaped crystals that are mainly visible at high power and may have a relatively localized distribution. The crystals typically appear pale with the hematoxylin and eosin and periodic acid Schiff stains, and often do not stain for κ or λ light chain by standard IF on frozen tissue. As a result, the diagnosis of LCFS may not be appreciated until ultrastructural evaluation, at which time abundant crystals of varying appearance are identified within the cytoplasm of proximal tubules. Interestingly, IF on formalin-fixed, paraffin-embedded, pronase-digested tissue appears to be superior to standard IF performed on frozen tissue for determining the light chain composition of the proximal tubular crystals and thus establishing the diagnosis of LCFS.[2] This may relate to the fact that pronase digestion has a denaturing effect on cell membranes, which may unveil sequestered antigenic sites.

LCFS is a relatively rare form of renal disease with fewer than 100 cases appearing in the medical literature, predominantly reported as isolated cases and small series. In 1975, Maldonado *et al.*[3] provided the first large series of LCFS, presenting 3 new cases from the Mayo Clinic and reviewing the findings in 14 previously published cases. In this initial description, common characteristics included relatively

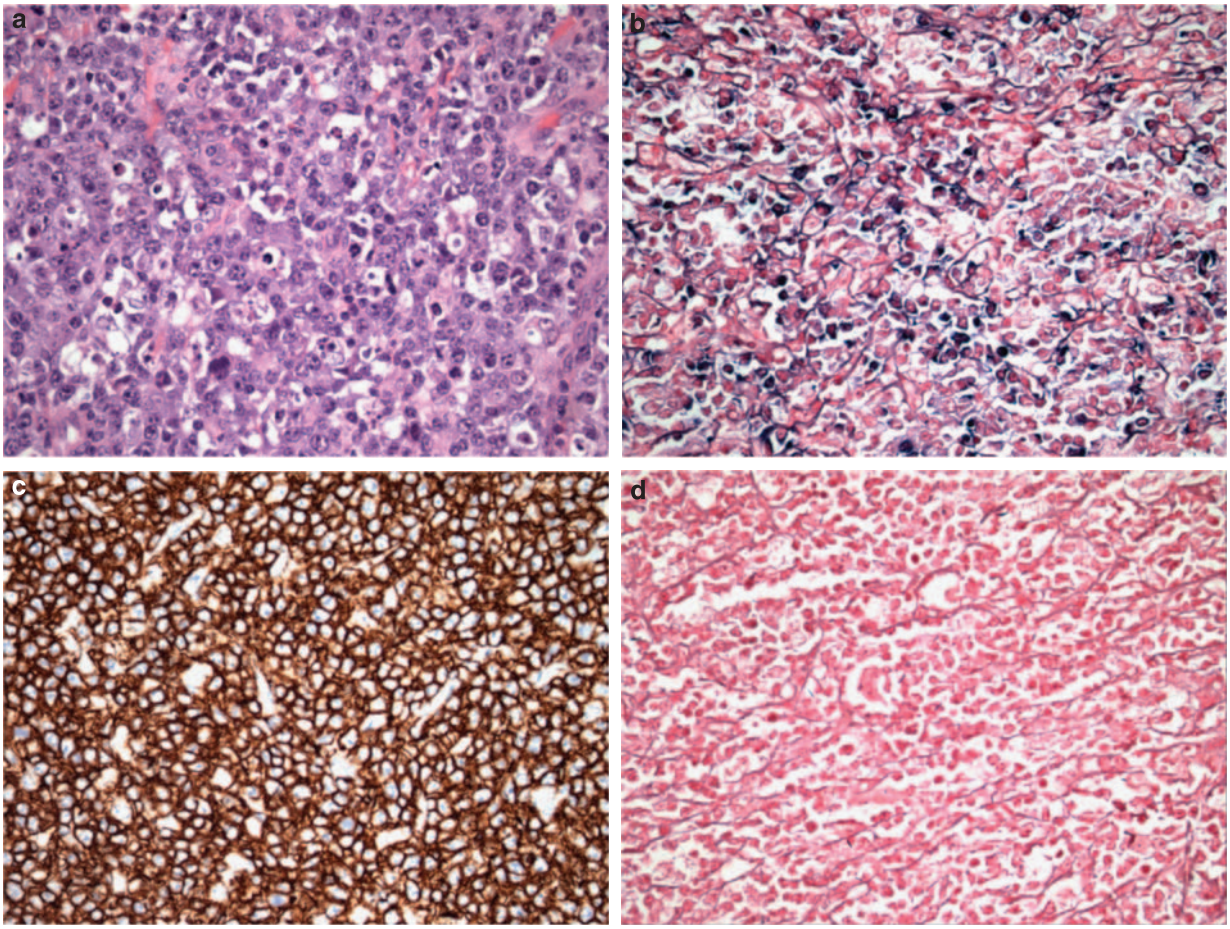

Figure 2 | Diffuse large B-cell lymphoma of the Spleen. (**a**) The splenic parenchyma is replaced by medium-to-large lymphocytes with round to ovoid nuclei, prominent nucleoli, and plasmacytoid morphology. Multiple apoptotic figures are seen (hematoxylin and eosin, ×400). (**b**) Immunohistochemical staining for CD20, a marker of B cells, is strongly positive in this diffuse large B-cell lymphoma (×400). (**c** and **d**) *In situ* hybridization of the neoplastic cells reveals diffuse κ light chain mRNA expression (**c**). *In situ* hybridization for λ light chain is negative (**d**) (×400, ×400).

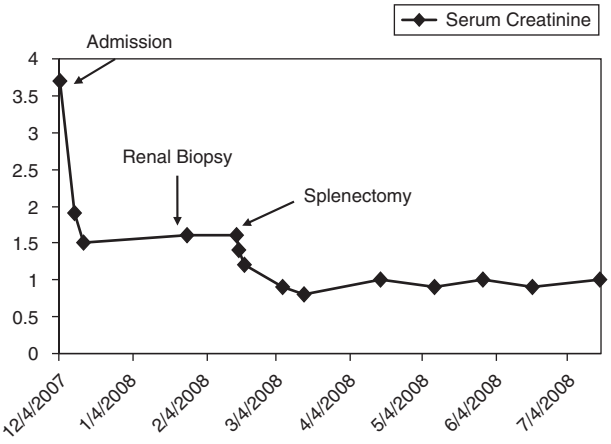

Figure 3 | Serum creatinine over time.

indolent renal dysfunction, Bence Jones proteinuria of κ-type, and in some cases, osteomalacia resulting from chronic hypophosphatemia. Of note, in 11 of the 17 cases LCFS was discovered before the development of MM or, less commonly, amyloidosis. In the remaining 6 patients, the diagnosis of LCFS and MM or amyloidosis was established simultaneously, leading the authors to suggest that LCFS represents a precursor to MM. In 2000, Messiaen *et al.*[4] detailed 11 cases of LCFS and emphasized the clinical heterogeneity of this entity. In this series, clinical presentations ranged from acute renal failure to more indolent renal dysfunction, a single patient presented with osteomalacia, and multiple patients came to clinical attention for bone pain that was subsequently found to relate to lytic lesions of MM. Features of proximal tubular dysfunction were identified in all 11 patients. In 2007, Kapur *et al.*[5] published a report of 5 cases of LCFS but broadened the definition to include 3 cases in which electron microscopy only revealed prominent phagolysosomes within tubular epithelia, which were subsequently found to contain κ light chains by immuno-electron microscopy.

Fanconi syndrome is uncommon in adults and most often signifies the presence of a plasma cell dyscrasia. A clinical diagnosis of FS or a pathologic diagnosis of LCFS should prompt a thorough hematologic work-up that may include serum protein electrophoresis, urine protein electrophoresis,

serum free light chain testing, bone marrow biopsy, and skeletal survey. The majority of reported cases of LCFS have occurred in patients who have evidence of or later develop MM or, less commonly, amyloidosis. There are also rare reports of patients with LCFS in the setting of chronic lymphocytic leukemia/small lymphocytic lymphoma.[6,7] In the case reported herein, LCFS was the result of κ light chain production by a large splenic mass of diffuse large B-cell lymphoma. To our knowledge, this is the first report of diffuse large B-cell lymphoma causing LCFS.

The propensity for the light chains in LCFS to form crystals that precipitate within the cytoplasm of proximal tubules appears to be determined by their amino acid sequences and the resultant physical and chemical properties. In the normal state, light chains pass through the GBM and are reabsorbed by the proximal tubule where they are broken down to amino acids by lysosomal enzymes. The first reported characterization of a monoclonal κ-cDNA from a patient with LCFS was published by Aucouturier *et al.* in 1993. Sequencing of the κ-cDNA showed that the variable region exons most closely fit into the VK1 subgroup. Protease treatment of the light chain produced an elongated NH2-terminal fragment of the variable (V) domain that was resistant to further degradation and was able to form crystals when bound to itself or intact κ light chains. It was hypothesized that the unique properties of this VK1 subgroup light chain, namely its resistance to complete proteolysis and its ability to form crystals, might explain the proximal tubular crystal formation and FS seen in patients with LCFS.[8] A subsequent study by the same group compared the protease resistance of light chains from 4 patients with LCFS to 12 patients with myeloma cast nephropathy. In all 4 patients with LCFS, the light chains were of the VK1 subtype and were partially resistant to proteolysis, producing a 12 kDa NH2-terminal fragment that was not further degraded by pepsin or cathepsin B, which are principle proteolytic lysosomal enzymes of the proximal tubules. In contrast, the light chains from 12 patients with myeloma cast nephropathy were susceptible to proteolytic digestion.[9] On the basis of these studies, the capacity to develop LCFS appears to be (1) intrinsic to the light chain molecule; (2) genetically determined as evidenced by common origin from the VK1 subgroup; and (3) based on a physical property of partial resistance to proteolysis, leading to the formation of a truncated NH2-terminal fragment with a propensity to crystallize. Analogous mechanisms of proteolytic resistance and crystal formation are likely to underlie the infrequent cases of LCFS associated with λ light chain production.[6,10]

In 2006, Sirac *et al.*[11] published a transgenic murine model of LCFS which supported many of the existing concepts on the pathogenesis of this condition. In this model, the mouse JK region was replaced by the VK-JK gene from a patient with LCFS. The mice developed LCFS with proximal tubular crystals that appeared similar to what has been described in the human condition, supporting the concept that the development of LCFS is determined by the amino acid sequence and resultant physicochemical properties of the monoclonal light chain. When the human VK-JK expression was conditionally deleted, tubular crystal formation was dramatically reduced.

Prognosis and optimal therapy for LCFS remain largely unknown. Although the majority of patients have relatively indolent renal dysfunction, more precipitous development of acute renal failure infrequently occurs. In the series of 11 patients reported by Messiaen *et al.*,[4] four patients reached the endpoint of doubling of creatinine or requirement for dialysis. In 2004, Ma *et al.*[12] reported the Mayo Clinic experience with adult-acquired FS. Over a 35-year period, 32 patients were seen including 10 with MM, 6 with smoldering MM, 2 with Waldenstrom's macroglobulinemia, and 14 with monoclonal gammopathy of undetermined significance (MGUS). The mean creatinine in this cohort was 2.0 mg/dl, all patients had evidence of light chain Bence Jones proteinuria (29 κ; 3 λ), and 69% had a monoclonal serum protein. Importantly, only 17 patients underwent renal biopsy, among which only 8 had crystals in proximal tubular cells characteristic of LCFS. Thus, the data from this cohort are reflective of a heterogeneous group that is not limited to individuals with LCFS. Nonetheless, the findings are interesting and noteworthy. Over the period of follow-up (mean 65 months), 5 patients progressed to ESRD, which in each instance was more than 7 years after the initial diagnosis of FS. The median time from diagnosis of FS to ESRD was 196 months, and only 1 of 10 patients with MGUS evolved to MM. Chemotherapy was given to all patients with MM or Waldenstrom's macroglobulinemia, 4 of 6 patients with smoldering MM, and 6 of 14 patients with MGUS. Among the 10 patients with smoldering MM or MGUS who received chemotherapy, no significant improvement in renal function was seen over 4–26 months of treatment. Importantly, 4 patients (1 MGUS, 1 smoldering MM, 2 MM) developed treatment-related myelodysplastic syndrome or acute leukemia and died. The authors concluded that the adult-acquired FS has a slow progression to ESRD and that the risk of chemotherapy with alkylating agents appears unjustified in patients who lack evidence of overt malignancy. Again, it must be pointed out that the majority of patients in this series did not have biopsy-proven LCFS.

In summary, LCFS is a rare disease characterized by slowly progressive renal dysfunction associated with FS. The diagnosis of LCFS requires pathologic evaluation, where the distinctive finding is accumulation of crystalline inclusions composed of light chains within the cytoplasm of proximal tubules. The monoclonal light chains are typically of κ type and are derived from the VK1 subgroup. These light chains have the properties of resistance to proteolysis within proximal tubules and a propensity to form intracellular crystals. Patients with LCFS typically have evidence of MM, 'smoldering MM,' or MGUS, although rarely this condition may be associated with B-cell non-Hodgkin lymphoma. Although limited data are available, in patients who lack evidence of overt malignancy, aggressive treatment with

alkylating agents does not appear justified. In cases of LCFS with malignancy such as the one reported here, long-term prognosis is likely to be closely tied to success in treating the underlying malignancy.

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